

Goal 1: Environmentally Friendly System

UNL iGEM 2016 *E. coli* serA kill switch in the Δ serA strain is environmentally friendly

- *Context 1: Environmental Hazards*
 - *Environmental hazards are specified in document X*
- *Context 2: Organism*
 - *UNL Δ serA strain of *E. coli**
 - *Non-pathogenic lab strain*
- Strategy 1.1: Argue over environmental hazards
 - Claim 1.1: Accidental release of UNL Δ serA strain into unintended environments is not harmful to the environment
 - *Context 1: The environmental impact of the UNL Δ serA strain's ability to reduce nitrates is out of the scope of this safety case*
 - Claim 1.2: Biomass from dead UNL Δ serA cells will not harm the environment
 - Claim 1.3: Bacteria conjugation will occur at an acceptably low level
 - Claim 1.3.1: The rate of bacterial conjugation in the UNL Δ serA strain is X
 - Claim 1.3.1.1: The probability of the UNL Δ serA strain to uptake a plasmid coding for serine is statistically insignificant
 - Evidence 1.3.1.1: The serA gene is typically located on chromosomal DNA in bacteria. (Eco Cyc)
 - Claim 1.3.2: The UNL Δ serA will likely only survive in the environment for Y (time)
 - Claim 1.4: Bacteria conjugation of the plasmid containing serA kill switch will not harm other bacteria
 - Claim 1.2.4: The synthesis of serA in other organisms is a natural process
 - Claim 1.5: The synthesis of serine will not harm the environment
 - Strategy 1.5: Argument by experimental results in literature
 - Claim 1.5.1: An abundance of serine in organisms is not harmful
 - *Context 1: serine is an amino acid found in (all?) organisms*
 - *Evidence 1: Mouse Serine Experiment*
- Strategy 2.1: Argue over programmed properties
 - Claim 2.1: Reliability: The serA kill switch will reliably work in the natural environment
 - *Context 1: Natural Environment:*
 - Claim 2.1.1: Nitrate ion concentrations in the environment will regulate the repression and induction of the *yeaR* promoter

- Strategy 2.1.1: Argument by appeal to test results
 - Claim 2.1.1.1: *yeaR* promoter is induced when nitrate concentrations are above X uM
 - Claim 2.1.1.2: *yeaR* promoter is repressed when nitrate concentrations are below X uM
 - *Context 1: Leakiness is minimal*
- Claim 2.1.2: The repression/induction of the *yeaR* promoter will regulate the expression of the *serA* gene
- Strategy 2.1.2: Argument by appeal to test results
 - Claim 2.1.2.1: serine synthesis exceeds the threshold to sustain life in the UNL Δ *serA* strain when the *yeaR* promoter is maximally induced
 - Claim 2.1.2.2: The UNL Δ *serA* strain requires an environment with a serine concentration of X to survive
 - Evidence 2.1.2.1: Literature: *E. coli* require 0.43 mM of serine to survive (Serine Metabolism in a Mutant strain of *E. coli* Strain K-12)
 - Claim 2.1.2.2: Serine synthesis is sub threshold to sustain life in the UNL Δ *serA* strain when the *yeaR* promoter is repressed
 - *Context 1: Leakiness of yeaR promoter*
 - UNDEVELOPED *We only have results for fluorescence of GFP, we cannot relate that to the production of *serA*
- Claim 2.1.3: The natural level of serine in the environment will not support life in the UNL Δ *serA* strain
- Strategy 2.1.3: Argument by appeal to test results
 - Claim 2.1.3.1: The UNL Δ *serA* strain requires an environment with a serine concentration of X to survive
 - Evidence 2.1.3.1: Literature: *E. coli* require 0.43 mM of serine to survive (Serine Metabolism in a Mutant strain of *E. coli* Strain K-12)
 - Claim 2.1.3.2: The concentration of serine in the intended marine environment is Y
 - Evidence 2.1.3.2: Literature: The environmental concentration of serine is 30 nM (Amino Acids Dissolved in Stream Water as Possible Home Stream Ordants for Masu Salmon)
- Claim 2.1.4: The UNL Δ *serA* strain will die in a reasonable time frame after *PyeaR* repression
 - *Context 1: A reasonable time frame refers to the cells dying before they deplete all environmental nitrate ions*
- Strategy 2.1.4: Argument by appeal to test results

- Claim 2.1.4.1: After PyeaR repression, the UNL Δ serA strain will die in X minutes.